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A clinical study of autologous chimeric antigen receptor macrophage targeting mesothelin shows safety in ovarian cancer therapy

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Abstract

CAR-macrophage has promising prospect in treating solid tumors, due to its high infiltration into tumors, and its dual roles in phagocytosis and immune modulation. Here we show the clinical results of CAR-macrophage treatment of two ovarian cancer patients. The CAR-macrophages were produced by introducing a mesothelin targeting CAR to patients' primary peripheral blood mononuclear cell-derived macrophages, and the products were infused to patients intravenously. Our data show good safety of the infusion product, and the efficacy can be further improved. Intraperitoneal infusion of CAR-macrophages has proven effective in treating intraperitoneal tumors in a preclinical model, paving the way for demonstrating proof-of-concept clinical efficacy of CAR-macrophages in the treatment of intraperitoneal tumors.

Keywords Chimera antigen receptor (CAR), Autologous macrophage, Mesothelin, Ovarian cancer

To the Editor,

Most ovarian cancer patients experience a relapse after initial surgery and chemotherapy [1]. The application of CAR-T cell therapy to solid tumors has proven challenging [2]. Due to the high infiltration rates and immune modulation capabilities [3], macrophages have emerged as a promising candidate for adoptive immune cell therapy in solid tumors. CAR-macrophages also demonstrate cross-presentation of intracellular tumor antigens, which may lead to antigen spreading, and a memory immune response, resulting in a durable anti-tumor effect [4]. In recent years, macrophages have been engineered to boost their phagocytic and cytotoxic activities against tumors, demonstrating effective antitumor activity in preclinical models [4–6]. Anti-HER2 CAR-macrophage CT-0508 (NCT04660929) and mRNA-based anti-mesothelin

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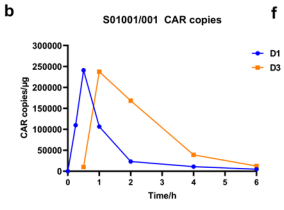
CAR-PBMC MCY-M11(NCT03608618) have exhibited safety in clinical trials. Thus, the potential of applying CAR-macrophages for treating solid tumors merits further exploration.

First, we utilized the Ad5f35 adenovirus for efficient transduction of mesothelin-targeting CAR (Fig. S1a-b) into human primary macrophages derived from patients, creating CAR-macrophages (CAR-pMAC) (Fig. S2a). Detailed materials and methods are provided in Supplementary Material 2. The CAR-macrophage product is designated as SY001. The purity of SY001 was assessed via flow cytometry. The analysis revealed that the proportion of $CD11b^+$ cells could be up to 99%, and the proportion of $CD14^+$ cells was approximately 80%. (Fig. S2b). We observed that following adenovirus transduction, macrophages underwent polarization towards the M1-like phenotype. Notably, the expression of CD80 was significantly heightened, while the expression of CD206 and CD163 were significantly decreased (Fig. S2c-e). This alteration is likely a consequence of the antiviral immune response [7]. To assess the anti-tumor efficacy, SY001 was co-cultured with SKOV3 and HO8910 ovarian cancer cells in vitro for 72 h. Following co-culturing, the tumor-killing efficiency reached to 80-100% (Fig. S2f). Moreover, the concentration of TNF-α in the co-culture medium of SY001 was significantly elevated compared to that in the WT-pMACs medium (Fig. S2g). Mesothelin expression on SKOV3 and HO8910 cells was comfirmed by flow cytometry (Fig. S3). These findings collectively suggest that SY001 is a product of M1-polarized CAR-macrophages with strong tumor-killing capabilities in vitro.

Next, two patients were recruited in a clinical study using SY001 for the treatment of advanced ovarian cancer. The baseline characteristics of their disease were recorded (Fig. 1a). The characteristics of SY001 used for the two patients are shown in Table S1. The treatment details are described in the Clinical Trial Information and Design section in Supplementary Material 2. The levels of CAR copies in the blood were assessed, with peaks observed in the range of 0.5 to 2 h following the administration of SY001 (Fig. 1b-c). Although all patients exhibited transient cytokine fluctuations and reductions in lymphocytes or neutrophils post-treatment, no other high-grade (≥3) adverse events or CRS were observed (Fig. 1d-f). Over the course of a 28-day follow-up period,

a

Subject number	Patient 1 (S01001/001)	Patient 2 (S01002/002)
Age (yr)	68	55
Gender	Female	Female
ECOG performance status	1	0
Mesothelin IHC 2+	85%	100%
Diagnosis	Bilateral ovarian cancer stage III C recurrence after three times postoperative chemotherapy (FIGO staging 2009)	Ovarian cancer stage IV recurrence after postoperative chemotherapy
Target lesions at baseline	82mm left axillary lymph nodes; 31mm retroperitoneal lymph nodes	18mm right supraclavicular lymph nodes; 15mm retroperitoneal lymph nodes
Non-target lesions at baseline	Multiple enlarged lymph nodes in the neck, supraclavicular region, left axilla, retroperitoneum of the abdomen, and pelvis	Strip-like shadows in the pelvic cavity, multiple lymph nodes of different sizes in the retroperitoneum and pelvic cavity



f

CellOrigin AE	Case number (n=2)			
	Grade 1	Grade 2	Grade 3	Total cases
Decreased lymphocyte count	1	0	3	4
Decreased white blood cell count	0	0	1	1
Decreased platelet count	1	1	0	2
Hypomagnesemia	1	0	0	1
Hypokalemia	0	1	0	1
Nausea	3	0	0	3
Fever	2	5	0	7
Elevated D-2 polymers	3	0	0	3
Headache	2	0	0	2
Chest distress	1	0	0	1
Chill	2	0	0	2
Decreased neutrophil count	0	0	1	1
Elevated CRP	2	0	0	2

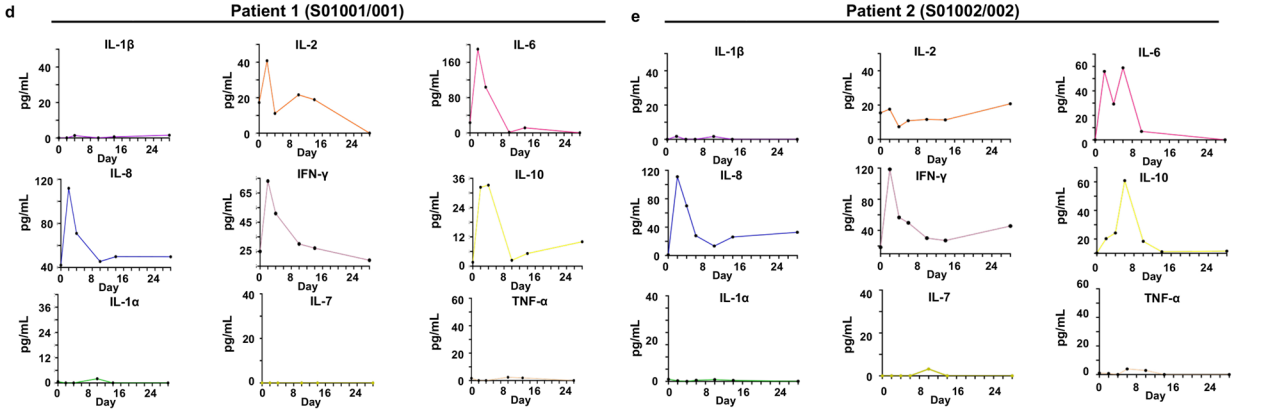


Fig. 1 SY001 CAR-pMACs showed safety in ovarian cancer patients. (**a**). Characteristics of patients at baseline. (**b and c**). CAR copy number in genomic DNA from the peripheral blood of patient 1 (**b**) and patient 2 (**c**) on the indicated time after infusion. (**d and e**). Changes in serum levels of CRS-related cytokines in patient 1 (**d**) and patient 2 (**e**) were measured, including interleukin (IL)-1β, IL-2, IL-6, IL-8, IFN-γ, IL-10, IL-1α, IL-7 and TNF-α. (**f**). Adverse events following SY001 infusion in patient 1 and patient 2.

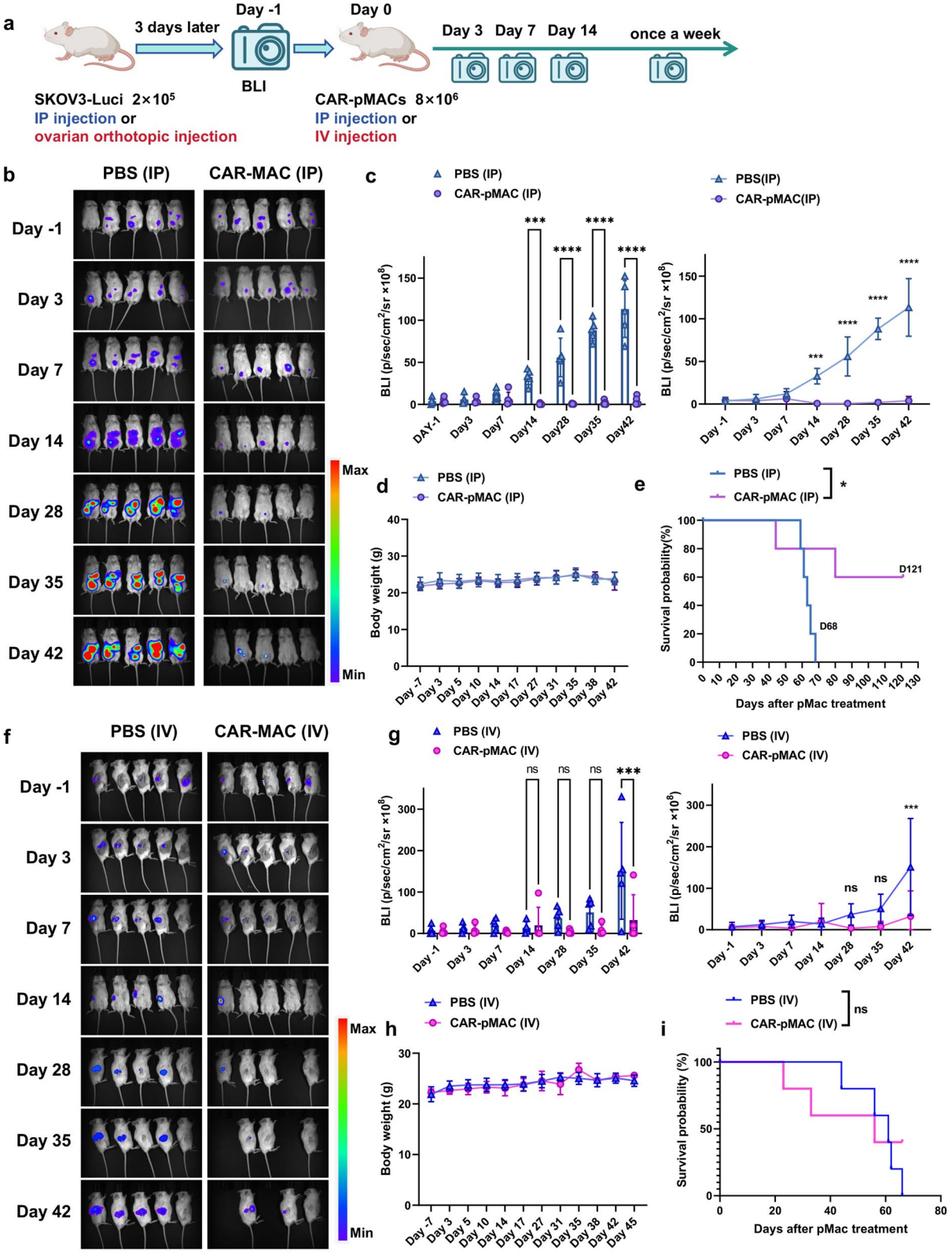


Fig. 2 (See legend on next page.)

(See figure on previous page.)

Fig. 2 Intraperitoneal infusion of CAR-macrophages is effective in treating intraperitoneal tumors in a pre-clinical model. (**a**). A diagram illustrating the in vivo treatment scheme for two different ovarian cancer models. (**b**). Images from the In Vivo Imaging System (IVIS) display the progression of tumors in the intraperitoneal (IP) model ($n=5$ per group). (**c**). Tumor burden in the IP model was quantified on day −1, 3, 7, 14, 28, 35 and 42 and the results were presented as mean ± SD ($n=5$ per group). Statistics by two-way ANOVA test. (**d**). The body weight of the mice in the IP model was monitored from day −7 to day 42. The data was displayed as mean ± SD ($n=5$ per group). (**e**). The Kaplan-Meier curve demonstrating survival of the mice in the IP model. Statistics by two-tailed log-rank test. (**f**). Images from the In Vivo Imaging System (IVIS) illustrate the progression of tumors in the intraperitoneal (IV) model ($n=5$ per group). (**g**). Tumor burden in the IV model was quantified on day −1, 3, 7, 14, 28, 35 and 42 and the results were presented as mean ± SD ($n=5$ per group). Statistics by two-way ANOVA test. (**h**). The body weight of the mice in the IV model was monitored from day −7 to day 45. The data was displayed as mean ± SD ($n=5$ per group). (**i**). The Kaplan-Meier curve demonstrating survival of the mice in the IV model. Statistics by two-tailed log-rank test. *$P<0.05$; ***$P<0.001$; ****$P<0.0001$; ns, not significant

both the two patients were at the SD status (Table S2). Collectively, these findings indicate that SY001 exhibit a high level of safety. Notably, the infusion product for patient 2 has a small portion of 8.87% T cells, suggesting that a mixture of CAR-macrophage and autologous T cells is also a safe product.

To delve into strategies for enhancing clinical efficacy, we conducted an assessment of the anti-tumor properties of SY001 across two distinct xenograft solid tumor models utilizing NSG mice. The detailed treatment scheme is shown in Fig. 2a and Supplementary Material 2. After this treatment, the mice were monitored using BLI. In the IP infusion model, administering SY001 significantly suppressed tumor progression, with most mice experiencing almost complete regression of the tumor burden (Fig. 2b-c). Moreover, there was no significant difference observed in body weight between the two groups (Fig. 2d). This antitumor efficacy resulted in a significant extension of survival time for the treated mice (Fig. 2e). In the IV infusion model, administration of SY001 only slightly suppressed tumor growth (Fig. 2f-g), without affecting the body weight (Fig. 2h). There was no significant difference of the survival time between the two groups (Fig. 2i). These data suggest that IP infusion of CAR-macrophages is effective in treating intraperitoneal tumors in a pre-clinical model, providing a proof-of-concept path toward improving clinical efficacy. In this study, we present the use of human primary CAR-macrophages for the treatment of two patients with advanced-stage recurrent ovarian cancer, demonstrating the safety profile of adenovirus-transduced autologous CAR-macrophage therapy for ovarian cancer. In our next clinical study, we plan to utilize IP administration of SY001 in combination with an anti-PD1 antibody in patients with high PD-L1 expression.

Abbreviations

NSG	NOD/SCID/IL2rγnull
CAR	Chimera antigen receptor
CRS	Cytokine release syndrome
SD	Stable disease
PBMC	Peripheral blood mononuclear cell
BLI	Bioluminescent imaging
IP	Intraperitoneal
IV	Intravenous

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s13045-024-01635-5.

Supplementary Material 1

Supplementary Material 2

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Author contributions

J.Z. and X.L. supported the study. X.W., L.L. and J.Z. drafted the manuscript. X.L., D.Z., and Y.F. conducted the clinical study. X.W., J.X., and D.X. carried out the pre-clinical experiments. H.W., X.W. and L.Z. were involved in the production of SY001.

Data availability

Data is provided within the manuscript or supplementary information files. The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate

The clinical protocol was reviewed and approved by the Clinical Research Ethics Committee of Linyi Cancer Hospital (2023IIT). The animal study was performed in compliance with relevant regulatory standards.

Competing interests

Jin Zhang is a scientific co-founder of CellOrigin. Hao Wang, Jianpo Xu, Yixuan Feng, Li Zhang, and Lin Lin are employees of CellOrigin. The remaining authors declare no competing interests.

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